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Nitrogen Balance in the Magruder Plots Following 109 Years in Continuous Winter Wheat#

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ABSTRACT

The Magruder plots are the oldest continuous soil fertility wheat research plots in the Great Plains region, and are one of the oldest continuous soil fertility wheat plots in the world. They were initiated in 1892 by Alexander C. Magruder who was interested in the productivity of native prairie soils when sown continuously to winter wheat. This study reports on a simple estimate of nitrogen (N) balance in the Magruder plots, accounting for N applied, N removed in the grain, plant N loss, denitrification, non-symbiotic N fixation, nitrate (NO_3^-) leaching, N applied in the rainfall, estimated total soil N (0–30 cm) at the beginning of the experiment and

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that measured in 2001. In the Manure plots, total soil N decreased from 6890 kg N ha^{-1} in the surface 0–30 cm in 1892, to 3198 kg N ha^{-1} in 2002. In the Check plots (no nutrients applied for 109 years) only 2411 kg N ha^{-1} or 35% of the original total soil organic N remains. Nitrogen removed in the grain averaged 38.4 kg N ha^{-1} yr^{-1} and N additions (manure, N in rainfall, N via symbiotic N fixation) averaged 44.5 kg N ha^{-1} yr^{-1} in the Manure plots. Following 109 years, unaccounted N ranged from 229 to 1395 kg N ha^{-1}. On a by year basis, this would translate into 2–13 kg N ha^{-1} yr^{-1} that were unaccounted for, increasing with increased N application. For the Manure plots, the estimate of nitrogen use efficiency (NUE) (N removed in the grain, minus N removed in the grain of the Check plots, divided by the rate of N applied) was 32.8%, similar to the 33% NUE for world cereal production reported in 1999.

Key Words: Nitrogen use efficiency; Manure; Wheat production.

THE MAGRUDER PLOTS

The Magruder plots have been managed under a conventional tillage production system since 1892. Each year, wheat straw residue is returned to the soil for decomposition via incorporation. Prior to being cultivated for the first time, the Magruder plots were native prairie grassland, recognized for its high organic matter levels and structural stability. The continual agricultural use of native prairie soils has resulted in significant reduction of soil organic matter levels,[1] as cultivation stimulates aerobic microbial activity, which in turn leads to increased decomposition of plant residue and an acceleration of the nitrogen (N) cycle. Also, incorporation tends to promote faster release of residue N than when residue is left on the surface.[2]

Over the past 109 years, several changes have been imposed on the original treatment structure set forth by A. C. Magruder. The most significant changes took place in 1929, when Horace J. Harper modified the Manure and Check plot combination to include a total of 10 treatments (five superimposed on top of the Check plot and Manure plots, respectively).

Six of the ten treatments evaluated in 1929 are continued today: (1) manure, (applied every four years); (2) check, no nutrients applied; (3) P, phosphorus applied each year; (4) NP, nitrogen and phosphorus applied each year; (5) NPK, nitrogen, phosphorus, and potassium applied each year; and (6) NPKL, nitrogen, phosphorus, and potassium applied each year +lime applied when soil pH < 5.5.

Added discussion and details associated with the Magruder plots can be found in Boman et al.[3] From 1892 to 1898, no fertilizer or manure was applied to the entire area. In the fall of 1898, these plots were split in two, half receiving 134 kg N ha^{-1} as beef manure every four years (1899–1967), and the check receiving no nutrient additions. From 1968 to present, 268 kg N ha^{-1} as beef manure has been applied every four years. From 1892 to 2001, winter wheat row spacings have ranged from 18 to 35 cm, and seeding rates have ranged between 56 and 84 kg ha^{-1}. From 1930 to 1967, the P source was ordinary super phosphate and since that time triple superphosphate has been used. The highest yielding hard red winter wheat varieties available at the time have been planted in the Magruder plots since 1913. In the early years of this trial, soft red winter wheat types were sown. Other management specifics associated with these plots are reported in Table 1.

NITROGEN MINERALIZATION AND NON-SYMBIOTIC FIXATION

Several factors contribute to the rate of N mineralization in the soil including moisture, temperature, microbial activity, texture, and time. Nitrogen

Table 1. Nitrogen application, source, and rate changes from 1892 to 2001, Stillwater, OK.

Practice	Manure	Check	P	NP	NPK	NPKL[d]
N fertilization, 1892–1898, kg N ha^{-1}	—	None	—	—	—	—
N fertilization, 1899–1929, kg N ha^{-1}	134[a]	None	—	—	—	—
N fertilization, 1930–1967, kg N ha^{-1}	134[a]	None	—	37[b]	37[b]	37[b]
N fertilization, 1968–present, kg N ha^{-1}	269[a]	None	—	67	67	67
P fertilization, 1930–present, kg P ha^{-1}	—	None	14.6[c]	14.6[c]	14.6[c]	14.6[c]
K fertilization, 1930–present, kg K ha^{-1}	—	None	—	—	28.8[e]	28.8[e]

[a]Applied once every four years.
[b]Inorganic N source was $NaNO_3$ (16-0-0) from 1930 to 1945, and has been NH_4NO_3 (34-0-0) from 1946 to present.
[c]P applied as ordinary superphosphate from 1930 to 1967, and as triple superphosphate from 1968 to present.
[d]6720 kg ha^{-1} of coarse limestone screenings applied in the fall of 1929, and 4480 kg ha^{-1} ground limestone per acre applied in 1954 (lime applied when soil pH is less than 5.5).
[e]K applied as muriate of potash (0-0-62) from 1930 to present.

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mineralization rates are highly variable both temporally and spatially. In Madrid, Spain, Sanchez et al.[4] found that for the soils under maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.), 2/3 of the whole available N during the growing season was mineralized from organic matter. Ma et al.[5] found that the amount of net N mineralized over a corn-growing season accounted for half the plant N uptake for all of the treatments in the experiment. During a three week period, net N mineralization was 10 kg N ha^{-1}, 16 kg N ha^{-1}, and 1 kg N ha^{-1} for soils fertilized with stockpiled manure, well-fertilized (200 kg N ha^{-1}), and control, respectively. Rasmussen et al.,[6] like Ma et al.,[5] found that mineralized N supplies large amounts (30–100%) of the nutritional N need of most non-legume crops. They reported that net N mineralization at 49 days was 18, 24, 29, and 57 mg N kg^{-1} soil for wheat-fallow, wheat–pea, continuous wheat, and grass pasture, respectively. Gil and Fick[7] found that in alfalfa (*Medicago sativa* L.) monoculture net mineralized N ranged from 35 to 100 kg N ha^{-1} yr^{-1}, gamagrass (*Tripsacum dactyleides* L.)–legume system ranged from 15 to 62 kg N ha^{-1} yr^{-1}, and gamagrass monoculture ranged from 2 to 15 kg N ha^{-1} yr^{-1}. Ledgard et al.[8] found that in three long-term grassland regimes (grass plus white clover, grass receiving 0 kg N ha^{-1} yr^{-1}, grass receiving 200 kg N ha^{-1} yr^{-1}), gross N mineralized was 4.8 μg N g^{-1} soil d^{-1} (0 kg N), 6.2 μg N g^{-1} soil d^{-1} (grass + white clover), and 6.2 μg N g^{-1} soil d^{-1} (200 kg N). In the northern Great Plains, Wienhold and Halvorsen[9] found that mineralization rates in response to different tillage, cropping, and nitrogen rates ranged from 2.3 to 22.9 μg N g^{-1} soil wk^{-1}. And finally, Tabatabai and Al-khafaji[10] measured N mineralization in 12 common Iowa soils and found that N release was linearly related to time with rates ranging from 7.7 to 17.0 μg N g^{-1} soil wk^{-1}.

Free-living microorganisms or organisms not directly associated with higher plants are capable of non-symbiotic N fixation.[11] Many heterotrophic bacteria are capable of fixing N including *Azotobacter* and *Beijerinckia* which are aerobes and occur in temperate and tropical soils, respectively. *Clostridium* is a heterotrophic bacterium that thrives only under anaerobic conditions. *Azospirillum* is a bacterium that has been found to live in the rhizosphere of the roots of tropical grasses. Certain photosynthetic bacteria and cyanobacteria ("blue-green algae") live near the soil surface and can fix N non-symbiotically.

For heterotrophic N-fixing microorganisms, organic carbon (C) is required as an energy source. The effect of organic matter on soil microbial activity depends on the type of material, its nutrient content, and on the initial fertility of the soil.[12] When organic materials such as sugars or straw are added to soil, N fixation can increase. Manure and fertilizer application resulted in higher concentrations of inorganic N capable of inhibiting dinitrogen gas (N_2) fixation and ultimately the presence/absence of these

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organisms.[13] Other work has suggested that NO_3-N > 35–40 kg ha^{-1} would inhibit N_2 fixation. As for autotrophic N-fixing micro-organisms, native fertility levels are important factors in their development. Nitrogen fixation by these organisms can approach 70 kg ha^{-1} yr^{-1}.[12]

In addition to affecting microbial activity, soil moisture affects gas exchange and the level of O_2 in the soil environment. Roper[14] found microbial activity appeared to be highest at or near field capacity, but fell sharply when the moisture level dropped to 80% field capacity. Low incubation temperatures, which simulated soil temperatures of tillage systems in the field, resulted in a lower fixation than at 25°C.[15]

Soil pH has a major influence on microbial activity in soil. In culture, diazotrophs tend to favor pH 7, although Azotobacter is more tolerant to high pH levels and Beijerinckia tolerates pH down to 5.0–5.5.[16] However, liming of acid soils has been found to stimulate both *Azotobacter* and *Beijerinckia* and increases N fixation.[12] It is generally accepted that the contribution of the nonsymbiotic N-fixing microorganisms to arable soils is small. For upland soils where wheat is grown, non-symbiotic N_2 fixation can approach 5 kg N ha^{-1} yr^{-1}.[17]

Benchmark Levels of Carbon and Nitrogen in Native Prairie Soils

The type of vegetation grown in an area affects and modifies the soil on which it grows. Post et al.[18] reported that the rate of soil organic C accumulation for grasslands is about 33.5 g of C m^2 yr^{-1}. If this accumulation rate is applied to the US land area converted from crop land into forests and grasslands over the past 50 years, the rate of organic C accumulation would be approximately 0.05 Pg C $year^{-1}$, a significant, fraction of the 1–2 Pg of C $year^{-1}$ rate of storage that has been inferred to be occurring for terrestrial ecosystems in the northern hemisphere.[18]

Rice et al.[19] in his comparison of the prairie site and wheat crop land, reported that microbial biomass C and N concentrations at the 0–5 cm soil depth ranged from 712 to 1165 μg C g^{-1} soil and 73 to 228 μg N g^{-1} soil for the prairie site, and 39 to 258 μg C g^{-1} and 40 to 68 μg N g^{-1} soil for the wheat site, respectively. He also reported that root biomass C concentrations ranged from 375 to 440 g C kg^{-1} biomass for the prairie site and from 355 to 389 g C kg^{-1} biomass for the wheat site. Historic soil test data from native prairies in North Dakota documented 35.8 to 45.8 tons C ha^{-1} of C in undisturbed soils.[20] Native prairie soils are good C sinks, but they have been altered by agricultural mechanization.[21] Agricultural soils, through cultivation, have been depleted of much of their original native C stocks.[22]

PATHWAYS FOR NITROGEN LOSS

Denitrification

Biogenic soil N emissions result from the second of two bacterial mediated processes: nitrification—the conversion of ammonium (NH_4^+) to nitrate (NO_3^-) and denitrification—the reduction of NO_3^- to N_2. The intermediate products of NO_3^- reduction, nitric oxide (NO) and nitrous oxide (N_2O), and the final reduced product of denitrification, N_2, are all easily lost from the soil[23] resulting in significant losses of plant available N from soil systems.

Gaseous losses of N from soil systems due to denitrification are influenced by numerous soil properties including soil water content, pH, and temperature,[24] but are largely controlled by the availability of water-soluble or readily decomposable organic matter and the lack of available oxygen.[25] In aerobic conditions, denitrifying bacteria use oxygen as their terminal electron acceptor. However, when oxygen becomes limited these facultative bacteria are able to use NO_3^- or nitrite as an alternative acceptor, thereby releasing N_2 into the atmosphere as biological oxidation of organic matter continues.

Quantification of biologically-derived gaseous N losses from the soil can be difficult due to small-scale variability in soil physical and chemical conditions, as well as inadequacies with current techniques to track gaseous N losses.[26] In addition, many studies have found soil N gas emissions are not only extremely heterogeneous spatially,[27–29] but temporally as well.[30,31] However, because of the importance of N in crop production, great efforts have been put forth to quantify gaseous N soil losses from crop production systems. In agricultural systems, gaseous N emissions have been shown to increase with increasing fertilizer applications.[32] Nitrogen losses of approximately 10–70% of the applied fertilizer N via denitrification have been documented in various cropping systems and climates.[23,24,33–36] These denitrification losses can increase to approximately 30–90% of applied fertilizer N when high amounts of organic residues are left on or added to the soil.[24,37–40] Specifically, Pu et al.[24] found denitrification losses of applied N increased with increasing residue; from 36–53% loss in fields with no added residues, to 59–79% loss in fields with low added residue, and 91–93% loss in soils with high rates of residue additions. The additions of crop residues are thought to increase denitrification rates by not only providing microbes with a decomposable carbonaceous substrate, but also by depleting oxygen in soil aggregates through the decomposition process.[24]

Yearly cumulative gaseous N losses from agricultural systems can be substantial even with moderate to little fertilizer additions. Jambert et al.[23]

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estimated gaseous N losses of 27–145.5 kg N ha^{-1} yr^{-1} from a cultivated maize field fertilized with 280 kg N ha^{-1}. Using the equation provided by Burford and Bremner,[25] soils containing 0.5–4% organic C could lose 31.2–206.2 μg N g $soil^{-1}$ yr^{-1}, respectively. This translates into a yearly loss of 78–515 kg N ha^{-1} due to denitrification assuming a 15.24 cm soil depth and a bulk density of 1.64 Mg m^{-3}.

Plant Nitrogen Loss

Historically, plant N loss has not been recognized as a significant factor in plant–soil system nitrogen use efficiency (NUE) calculations.[41] However, scientists have recently documented that cereal plants release significant amounts of N from plant tissue.[42,43] As N balance calculations are typically made at maturity, the effects of vegetative N losses on N balance calculations are typically not emphasized and may result in overestimation of denitrification, leaching, and ammonia volatilization.[43]

The N concentration of plant tissue has been observed to decrease during the growing season as N assimilation rates decrease relative to plant C as the plant matures.[44] Significant losses of volatilized NH_3 have also been noted during the early grain-filling period immediately after anthesis, resulting from inefficient N translocation and reassimilation within the plant.[42,44,45] In addition, plant N losses are generally higher at elevated levels of soil N, increasing the concerns of NUE in high yield agriculture.[44,46]

Many studies have been conducted to quantify the amount of NH_3 lost from various crops. Stutte et al.,[47] estimated that as much as 45 kg N ha^{-1} yr^{-1} was lost from soybean [*Glycine max* (L.) Merr.]. Plant N losses in corn (*Zea mays* L.) increased with elevated N fertilizer rates and accounted for 45–81 kg N ha^{-1} yr^{-1} of the unaccounted N loss.[43] Harper et al.[42] found that volatilized NH_3 loss from wheat (*T. aestivum* L.) accounted for as much as 21% of applied fertilizer N. In addition, Daigger et al.[44] found that N loss from anthesis to maturity was 25 kg N ha^{-1} yr^{-1} from non-fertilized wheat increasing to 80 kg N ha^{-1} yr^{-1} from wheat receiving 150 kg N ha^{-1}. In a two-year study conducted in Oklahoma, Kanampiu et al.[45] found that plant N loss (7.7–31.4 kg N ha^{-1} yr^{-1}) from wheat was greater at high N rates.

Nitrate Leaching

Quantification of NO_3^- formation and its fate in the soil is a fundamental part of a total N mass balance.[48] One of the fates of NO_3^- in the soil involves its downward movement in the soil beyond the root zone, entering the ground water where it becomes a concern to water quality. For NO_3^- to escape beyond the root zone two basic conditions need to be fulfilled:[49,50] (1) the soil permeability must allow downward movement of water, and (2) there must be sufficient rainfall to move NO_3^- downward. Brouder and Joern[51] reported that 2.5 cm of rainfall move NO_3^- to 30 cm. They further indicated that for NO_3^- to move beyond the root zone at least 183 cm of rainfall is required in light soils.

The amount of NO_3^- subject to leaching has been extensively studied for specific cropping systems and soil conditions in different parts of the world (Table 2). Most of these investigations focused on quantifying the amount of NO_3^- leached from addition of N fertilizer. Other findings indicated that NO_3^- leaching occurs only when fertilizer N is applied in excess of that amount required for optimum crop growth.[58] Studies conducted at Oklahoma State University have also suggested that no N leaching will be expected from fertilizer rates below optimum crop requirement.[59,60] It is imperative thus to carefully calculate the leaching loss of NO_3^- where no fertilizer or manure input have been applied for the last 109 years in the Magruder plots.

NITROGEN BALANCE IN THE MAGRUDER PLOTS

Results from soil samples taken from the Magruder plots in February of 2002 (20 cores per plot) are reported in Table 3. Data from analyses of NH_4-N, NO_3-N, P, K, organic C, total N, and pH are included for both the 0–15 and 15–30 cm sampling depths. Table 4 reports soil organic matter levels from 1892 to 2002 for all treatments in years where data was recorded for samples taken from the surface 0–15 cm.

Using the value for soil organic matter reported by A. C. Magruder in 1892 as the benchmark level, decreases in soil organic matter, by treatment are

Table 2. Estimates of NO_3-N leaching losses from various cropping systems.

Cropping system	Nitrate leached ($kg\,ha^{-1}\,yr^{-1}$)	Reference
General	2–100	[52]
Spring barley, unfertilized plot	83	[53]
Continuous corn, unfertilized, 3-year average	27	[54]
Barley, unfertilized	5	[55]
Continuous winter wheat, unfertilized	15–18	[56]
Winter wheat, continuous	17–28	[67]

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Table 3. Results from surface soil (0–15 cm) samples taken from the Magruder plots in February, 2002.

Treatment	Depth (cm)	NH_4-N (mg kg^{-1})	NO_3-N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Organic C (%)	Total N (%)	C : N	pH
Manure	0–15	6.85	2.15	29.05	271.75	0.867	0.068	12.8	6.35
	15–30	6.74	3.2	10.09	167.00	0.729	0.063	11.6	6.39
Check	0–15	4.87	0.01	6.98	149.15	0.586	0.049	12.0	5.50
	15–30	5.81	2.94	3.67	127.85	0.586	0.050	11.7	5.65
P	0–15	6.17	0.62	40.03	138.60	0.645	0.053	12.6	5.33
	15–30	4.85	0.13	16.00	127.60	0.603	0.055	10.9	5.59
NP	0–15	6.65	1.61	37.75	187.45	0.765	0.070	10.9	4.79
	15–30	6.55	7.47	9.16	133.30	0.710	0.058	12.4	5.31
NPK	0–15	7.87	2.3	38.48	248.35	0.759	0.072	10.6	4.70
	15–30	5.21	7.01	9.47	167.70	0.695	0.060	11.6	5.36
NPKL	0–15	7.39	1.36	36.51	250.05	0.791	0.068	11.7	4.95
	15–30	6.38	6.12	8.33	163.50	0.715	0.059	12.2	5.87

Note: NH_4-N and NO_3-N—2 M KCL extract; P and K—Mehlich-3 extraction; Organic C and Total N—dry combustion; pH—1 : 1 soil : deionized water.

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Table 4. Changes in surface (0–30 cm) soil organic matter levels in the Magruder plots, 1892–2002, Stillwater, OK.

	Organic matter (%)					
Crop year	Manure	Check	P	NP	NPK	NPKL
1892	3.58	3.58	3.58	3.58	3.58	3.58
1926	2.68	1.85	NA	NA	NA	NA
1938	2.32	1.69	1.77	1.65	1.64	1.70
1954	1.76	1.35	NA	NA	NA	NA
1978	1.54	1.18	NA	NA	NA	NA
1990	2.15	1.71	1.92	1.97	2.16	2.20
1991	2.15	1.71	1.92	1.97	2.17	2.20
1992	2.13	1.77	1.82	1.99	2.44	2.15
1993	2.12	1.76	1.82	1.99	2.44	2.14
1994	2.44	1.84	2.04	2.33	2.11	2.34
1995	2.56	1.99	2.15	2.42	2.20	2.56
1996	2.52	1.68	1.71	2.00	2.17	2.36
1997	2.40	1.48	1.71	1.98	2.00	2.09
2001	1.49	1.26	1.24	1.49	1.53	1.81
2002	1.60	1.17	1.25	1.48	1.45	1.51
OM decrease	1.98	2.41	2.33	2.10	2.13	2.07
Total (%)	55	67	65	59	59	58

Note: All years where organic matter levels were recorded are included. 1990–present: organic matter calculated by multiplying organic C in percent by 2.

estimated in Table 4. As is noted, organic matter levels decreased 55–67% depending on treatment. These results suggest that of the six treatments, application of manure resulted in the least amount of soil organic matter loss (note that manure is only applied every four years and was last applied in 1999). These results also indicate that most of the soil organic matter was lost in the first few years of cultivation. Although manure and supplemental N, P, K treatments are beneficial, they have not maintained organic matter levels in the soil and other measures must be taken to maintain and/or increase soil organic matter.

Using the 3.58% OM reported by A. C. Magruder, a bulk density of 1.45 g cm^{-3} (National Soil Survey Characterization database, http://vmhost.cdp.state.ne.us:96/SSSNB.html) and a C/N ratio of 11.6, initial total soil N in the 0–30 cm profile was estimated to be 6890 kg N ha^{-1}. These were estimates for an undisturbed Kirkland silt loam (fine, mixed, thermic Udertic Paleustoll), found in a nearby cemetery known to have never been tilled.

Table 5. Estimates of grain N removed in the Magruder plots from 1892 to 1929, and 1930 to 2001, Stillwater, OK.

Crop year	Manure	Check	P	NP	NPK	NPKL
Grain N removed, kg ha^{-1} 1892–1929	1,191	761	761	761	761	761
Grain N removed, kg ha^{-1} 1930–2001	2,995	2,047	2,183	3,214	3,229	3,330
Total, kg ha^{-1}	4,186	2,808	2,944	3,975	3,990	4,091
Average per year, kg ha^{-1}	38.4	25.7	27.0	36.5	36.6	37.5

Note: From 1892 to 1929, there were two plots (Check and Manure). The Check, P, NP, NPK, and NPKL plots were split from the original Check where no N had been applied, thus the same grain N removed is reported for all these plots from 1892 to 1929.

Total grain N removed after 109 years of continuous wheat production in the Magruder plots is reported in Table 5. These estimates come from multiplying actual grain yield by percent N in the grain. In years where percent N was not analytically determined, a fixed value of 2.28% N in the grain was used. As reported by Gauer et al.[61] and Halvorson et al.[62] increasing N level (manure or inorganic N fertilizer) resulted in increased grain N removal. The average grain N uptake ranged from 25.7 to 38.4 kg ha^{-1} yr^{-1}, consistent with long-term results reported by Bauer et al.[63]

In terms of N balance, grain N removal can be reliably estimated (as reported in Table 5) from the Magruder plots over this 109-year period because we have complete yield records from all these plots and reasonable data for grain N concentration. Also, N applied as fertilizer is well known, because records of the amounts and sources used have been meticulously recorded over time. The rates have varied somewhat as a function of production levels, but this has not affected the reliability for which N balance can be reported (Table 1). However, the other components of N balance from these plots are much more cumbersome and require several assumptions and reliance on previously published data that may or may not be consistent with winter wheat production in the Central Great Plains region. Albeit that these problems exist, a summary of the sources of N addition and removal is reported in Table 6.

When these native prairie soils were first tilled, A. C. Magruder reported that the soil organic matter level was 3.58%. While many scientists do not concur on the effect of fertility applications (organic or inorganic) on the C : N ratio in soils, they do agree that the C : N ratio of most virgin prairie soils

Table 6. Estimates of total amounts of N applied and removed from the Magruder plots from 1892 to 2001, Stillwater, OK.

N source/sink	Manure	Check	P	NP	NPK	NPKL
Total soil N in organic matter, 1892, kg ha^{-1}	6,890	6,890	6,890	6,890	6,890	6,890
N applied from rainfall, kg ha^{-1}	545	545	545	545	545	545
N fertilizer applied, kg ha^{-1}	4,200	0	0	3,321	3,321	3,321
N from non-symbiotic N fixation[a] kg ha^{-1}	109	218	218	146	146	146
Additions + initial total	11,744	7,653	7,653	10,902	10,902	10,902
N removed in the grain, kg ha^{-1}	4,186	2,808	2,944	3,975	3,990	4,091
Estimated plant N loss, kg ha^{-1}	1,726	1,252	1,252	1,614	1,614	1,614
Soil denitrification, kg ha^{-1}	769	265	265	664	664	664
Nitrate-N leaching losses, kg ha^{-1}	470	306	306	436	436	436
Removal (loss) total	7,151	4,631	4,767	6,689	6,704	6,805
Total soil N in organic matter, 2002, kg ha^{-1}	3,198	2,411	2,657	3,149	3,247	3,124
Total unaccounted	1,395	611	229	1,064	951	973
kg N unaccounted yr^{-1}	13	6	2	10	9	9

[a]Includes an added 37 kg N ha^{-1} for the 37 years where no N had been applied, from 1892 to 1929, assuming 2 kg N ha^{-1} yr^{-1} fixed via non-symbiotic N in the 0-N plots and 1 kg N ha^{-1} yr^{-1} where N was applied.

was approximately 10 : 1.[64–67] At 3.58% organic matter, a C : N ratio of 10 : 1, and a bulk density of 1.45 g cm^3, this would translate into 6890 kg of N in the 0–30 cm profile in 1892 when the Magruder plots were tilled for the very first time.

For the central region of Oklahoma, the National Atmospheric Deposition Program[68] reports average wet deposition of N from NO_3^- and NH_4^+ to range somewhere between 4 and 5 kg N ha^{-1}. This is consistent with average chemical rain gauge data reported by Sharpley et al.[69] at El Reno (60 miles south west of the Magruder plots) of 5.54 kg N ha^{-1} yr^{-1}. Using an average of 5 kg N ha^{-1} yr^{-1} deposited in the rainfall, this resulted in a total addition of 545 kg N ha^{-1} (Table 6).

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Nitrogen applied from manure and as inorganic N ranged from 3321 (NP, NPK, and NPKL) to 4200 kg N ha^{-1}. These would have been the same, but the Manure plots received N from 1892 to 1929, whereas the NP, NPK, and NPKL plots were derived from a larger "Check" that received no N for the first 37 years. Since 1929, the NP, NPK, NPKL, and Manure plots have received the exact same amount of total N applied. Nitrogen addition via non-symbiotic N fixation was estimated to be 1 kg N ha^{-1} yr^{-1} for plots receiving N fertilizer and 2 kg N ha^{-1} yr^{-1} in plots receiving no N.

Total N losses via denitrification over the past 109 years were calculated by combining the unfertilized soil denitrification estimates calculated using Burford and Bremner[25] with the estimated denitrification rate of spring applied fertilizer to wheat.[35] All calculations were based on a constant bulk density of 1.64 g cm^{-3} over an area of 0.02 ha to a depth of 15.24 cm. Where soil organic matter contents were available, total C contents were calculated using the conversion equation of Ranney.[70] Using these research reports, denitrification losses ranged between 265 and 769 kg N ha^{-1} over the 109-year period.

Plant N loss was calculated using previously published work by Kanampiu et al.[45] on winter wheat in this same area. Using a 12 kg N ha^{-1} yr^{-1} estimate of Kanampiu et al.,[45] 1308 kg N ha^{-1} would be lost over a period of 109 years, however, this would not have been accurate for all plots since different total N rates were applied over this 109-year period. By correlating plant N loss with applied N rate from Kanampiu et al.,[45] a linear regression equation was developed to quantify the relationship between plant N loss and applied N rate whereby plant N loss $= 0.1098 *$ applied N rate $+11.49$. Using this equation, plant N losses ranged from 1252 to 1726 kg N ha^{-1}.

Since conditions favoring leaching have occurred in the Magruder plots, we contemplated using the 5 kg ha^{-1} yr^{-1} leaching losses suggested by Bergström.[55] Using this estimate, the total amount of N leached would have been 545 kg N ha^{-1} over the 109-year period. However, we wanted to consider total N loading as this would be expected to influence the total amount lost via leaching. Thus, work by CSIRO[71] was applied here indicating that 4% of the total amount present and/or applied (total soil N, rainfall additions, non-symbiotic N, and fertilizer N). Using this value, leaching losses ranged from 306 to 470 kg N ha^{-1} (Table 6).

Nitrogen remaining in the soil organic matter was estimated by collecting comprehensive soil samples (0–15 and 15–30) from all plots and determining organic C and total N by dry combustion.[72] Total N in kg ha^{-1} in the 0–30 cm profile in 2002 was computed using a bulk density of 1.64 g cm^3, determined from measurements taken within the "Check" plot. This is much higher than the bulk density value used for this Kirkland silt loam when it was first cultivated (1.45 g cm^3). Total soil N in the surface 0–30 cm ranged from 2411 to 3247 kg ha^{-1} for samples collected in 2002. Over the 109 years, this represented a decrease in soil organic N from the original 6890 kg N ha^{-1} of up to 4479 kg ha^{-1} (Check, Table 6).

By subtracting the sum of N removed in the grain, plant N loss, soil denitrification, NO_3^- leaching, and total soil N in 2002, from the sum of total soil N in 1892, N applied in rainfall, N applied from fertilizer, and non-symbiotic N fixed, a balance by treatment is reported in Table 6. The total amount of N unaccounted for using these inputs ranged from 229 to 1395 kg N ha^{-1} over the 109 years included in this work. The largest amount of unaccounted N was recorded for the Manure plots which have also received the highest amount of applied N. As was indicated in methods, the Manure plot was the only one receiving any kind of applied N for the first 37 years, and as a result, total N loading was higher. In the two plots that have never received applied N, the total amount unaccounted was less in the plot receiving added P than in the check (no nutrients applied over this 109 year period). Total amounts of N removed in the grain were similar for these two plots, as were estimates of NO_3-N leaching, plant N loss, denitrification, and non-symbiotic N fixation. Thus the major differences were in the final estimate of total soil N in the 0–30 cm profile.

Because of the complexity of a 109-year-old continuous wheat production system, dependent upon yearly environmental changes that influence each of the parameters estimated or predicted in this work, we did not expect to arrive at an N balance of zero. Accounting for the entire N in this system would have required incredibly sophisticated monitoring at the onset of the trial in 1892. Obviously this was a technological impossibility in 1892, and to some extent is not functionally possible today. Table 6 meets the objectives of our work, providing an estimate of N balance in a long-term experiment where N has been applied and removed for 109 years. If more N was applied, more N was unaccounted for. As a percent of the total (additions + initial amount present in the profile), unaccounted N ranged from 2.9 to 11.8%. Considering the time spanned, variety changes, influence of the environment, and the errors associated with estimated additions and losses over the years, the N balance values reported here should be used with some caution.

For the Manure plots (109 years), the estimate of NUE (N removed in the grain, minus N removed in the grain of the check plots, divided by the rate of N applied) was 32.8%. This is consistent with values of 33% reported by Raun and Johnson[41] for cereal grain production worldwide. Over the last 71 years (1930–2001), NUE in the Manure, NP, NPK, and NPKL plots was 29, 35, 36, and 39%. It is not entirely clear why the NUE in the NPKL plot was

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higher compared to that reported for the Manure plot, however, this could be due to the inefficiency of applying N every four years (Manure) vs. every year (NPKL).

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